## 97
## BLOOD SERUM TO DIAGNOSE OSTEOARTHRITIS – BIOMARKERS AND MACHINE LEARNING

B.J. Heard, J.M. Rosvold, M.J. Fritzler, H. El-Gabalawy, J.P. Wiley, R.J. Krawetz. *Univ. of Calgary, Calgary, AB, Canada*

**Purpose**: Although reliable diagnostics for rheumatoid arthritis (RA) exist, currently only relatively advanced osteoarthritis (OA) can be clinically diagnosed with high confidence using a combination of physical exam / functional assessment with radiographic evidence of OA related structural changes (e.g. joint space narrowing, osteophytes). The objective of this study was to develop a method for categorizing patients with OA and RA, as well as discriminating both groups from normal individuals based on a panel of inflammatory cytokines expressed in blood serum samples.
**Methods**: Inclusion criteria required a diagnosis of mild/moderate OA performed by a sports medicine physician at the University of Calgary based on clinical symptoms for $\geq$ 3 months with radiographic evidence of changes associated with OA. Inclusion for RA patients required physician diagnosis (autonuclear antibody positive). RA patients were all receiving various anti-inflammatory treatments at the time serum was sampled. Normal control individuals were disqualified if any personal or family history of arthritis or autoimmune diseases were disclosed (e.g. systemic lupus erythematosus, sclero- derma, polymyositis, vasculitis, spondyloarthropathies, inflammatory bowel disease, diabetes mellitus type I, and/or thyroid disease). Blood serum was collected and a panel of 38 inflammatory proteins was quantified using Luminx Multiplexing. This panel was then used as a training set in the construction of an artificial neural network (ANN) (Matlab – ANN toolbox) to indentify patients with OA ($n$ = 100), RA ($n$ = 100), and normal individuals ($n$ = 100). The results for the ANN were verified by reexamining the data using a decision tree algorithm (Matlab – Treebagger Algorithm).
**Results**: High levels of sensitivity and specificity were obtained within the ANN using all 38 cytokines (sensitivity/specificity – Norm: 100/100, OA: 100/100, RA: 100/100). These results were verified through the reinvestigation of the data set using a decision tree algorithm that returned a similar sensitivity/specificity (Norm: 100/96, OA: 100/97, RA: 95/100). Results have been summarized in Table 1, this table shows a summary of the ANN (right) training, validation, and test sets, as well as the decision tree (left) training, test, and all data sets. Data is presented as the number of patients allocated by patient type on the left, and the diagnosis (categorization by the algorithm) on the top.
**Conclusion:** Using the panel of 38 cytokines, we were able to train an ANN to distinguish between our 3 patient groups with a high degree of sensitivity and specificity. These results were verified using a decision tree algorithm. Joint imaging techniques are improving in the diagnosis of OA, but the need still exists for a reliable non-invasive diagnostic tool to at least support an early and accurate diagnosis. Through the use of a panel of well documented inflammatory cytokines, we have developed a non-invasive test that can be used to identify patients with OA of the knee. Although there is currently no disease modifying treatment for early OA, however, the panel of cytokines that we have developed may prove useful for the development and evaluation of future pharmacological or even surgical interventions. Osteoarthritis is a complex disease with a complex phenotype, to account for heterogeneity of a patient population in addition to heterogeneity of a disease state (e.g. onset, pathogenesis and progression) an effective diagnoses will likely require a relatively complex interpretation of data.

**Table 1**
ANN and decision tree summaries

| ANN: All Cytokines (n=38) | | | | | Treebagger: All Cytokines (n=38) | | |
|---|---|---|---|---|---|---|---|
| | | Diagnosis | | | Diagnosis | | |
| | | Normal | OA | RA | Normal | OA | RA |
| **Training Set** | | | | | **Training Set** | | |
| Patient Type | Normal | 75 | 0 | 0 | 76 | 0 | 0 |
| | OA | 1 | 66 | 0 | 0 | 71 | 0 |
| | RA | 1 | 0 | 67 | 0 | 0 | 63 |
| **Validation Set** | | | | | **Test set** | | |
| Patient Type | Normal | 11 | 0 | 0 | 24 | 0 | 0 |
| | OA | 0 | 21 | 0 | 0 | 29 | 0 |
| | RA | 0 | 0 | 13 | 1 | 1 | 35 |
| **Test Set** | | | | | **All Data** | | |
| Patient Type | Normal | 12 | 0 | 0 | 100 | 0 | 0 |
| | OA | 0 | 13 | 0 | 0 | 100 | 0 |
| | RA | 0 | 0 | 20 | 1 | 1 | 98 |

## 98
## INCREASED ADIPOSITY IN RESPONSE TO HIGH FAT/HIGH SUCROSE DIET IS ASSOCIATED WITH INCREASED CONCENTRATIONS OF CYTOKINES AND ADIPOKINES IN SYNOVIAL FLUID

K.H. Collins, R.A. Reimer, W. Herzog. *Univ. of Calgary, Calgary, AB, Canada*

Obesity is associated with chronic inflammation. The increased incidence of Osteoarthritis (OA) in obese individuals has been attributed to increases in mechanical joint load. However, a distinct subtype of OA may be the result of other intrinsic factors related to adiposity. Therefore this so-called “metabolic” OA displays a unique disease trajectory, potentially independent of joint loading. Sprague Dawley rats display diet-induced obesity prone (DIO-P) and obesity resistant (DIO-R) phenotypes, providing the opportunity to evaluate increased adiposity when mass is conserved.
**Purpose**: The purpose of this study was to compare the joint damage and synovial fluid (SF) biomarkers from DIO-P and DIO-R rats after six months of high fat diet.
**Methods**: Fourteen male, 8-12 week old Sprague-Dawley rats were given a high fat diet (40% fat) for 12 weeks, at which point the top 50% percent of weight gainers (based on % change in mass over 12 week obesity induction) were labeled DIO-P and the bottom 50% were labeled DIO-R. Rats were followed for an additional 16 weeks continuing the same high fat diet. At sacrifice, body composition (% body fat, BF) was measured using Dual Energy X-ray Absorptiometry. Twenty seven serum and SF cytokines and adipokines were quantified using a Luminex®xMAP Rat 27 Multiplex Discovery Assay, and urea was quantified in both serum and SF using an ELISA (Eve Technologies, Calgary, AB). SF and serum concentration of urea was used to adjust for differences in SF concentration. Joints were harvested and scored on a 14-point Mankin scale in five regions along the joint (medial tibial plateau, lateral tibial plateau, medial femoral condyle, lateral femoral condyle, and patella). Subchondral bone and synovium were assessed using each respective (5- and 6- point) criteria from the rat OARSI metric. A Modified Mankin composite score was determined by adding the 7 scores to provide a volumetric quantification of damage. Kruskal-Wallis tests and Pearson correlations were used to evaluate Modified Mankin Composite scores, and to associate SF and serum cytokines by obesity response ($\alpha = 0.1$).
**Results**: Body Mass was not different between the groups (DIO-P= 729 $\pm$ 43g DIO-R= 792 $\pm$ 48g, $p = 0.18$). Percentage body fat was higher in DIO-P (41.8 $\pm$ 2.4%,) compared with DIO-R (32.5 $\pm$ 0.8%,$p = 0.002$). OA scores were higher in DIO-P (DIO-P mean= 27, 95% CI (17-37), DIO-R mean= 18, 95% CI 8-30, $p = 0.09$). DIO-P OA scores were associated with body fat percentage (r = .81, $p = 0.02$), and 6 SF markers: leptin (r = .68, $p = 0.08$), IL-5 (r = 0.63, $p = 0.09$), IL-18 (r = .74, $p = 0.03$) IP10/CXCL10 (r = .74, $p = 0.02$), VEGF (r = .74, $p = 0.03$) and RANTES/CCL5 (r = .72, $p = 0.04$). Serum markers associated with DIO-P OA scores include: IL-1$\beta$ (r = 0.744, $p = 0.03$), IL-10 (r = .66, $p = 0.07$), IP10/CXCL10 (.844, $p = 0.01$). IP10/CXCL10 serum concentration was strongly associated with IP10/CXCL10 SF concentration (r = .78, $p = 0.02$), but neither Il-10 nor IL-1$\beta$ serum was associated with their respective SF concentration. None of the measured serum or SF markers were associated with OA score in DIO-R animals.
**Conclusion**: DIO-P animals had increased joint damage compared to DIO-R. Increased adiposity is associated with increased Modified Mankin Composite score in DIO-P animals. Body fat and six pro-inflammatory cytokine SF concentrations were associated with OA damage in DIO-P animals. Most notably, IP10/CXCL10 (Interferon-gamma inducible protein 10) is associated with joint damage in both SF and serum concentrations, and there is a strong relationship between SF and serum concentration of this chemokine. IP10/CXCL10 is a chemoattractant for monocytes and macrophages, selectively activated by leptin, which has been implicated in cartilage damage. IP10/CXCL10 is associated with angiogenesis, and subcutaneous injection of this chemokine demonstrates increased infiltration of monocytes in fat and muscle. Further investigation is needed to understand the role of IP10/CXCL10 in metabolic OA.